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CLAIMS

What is claimed is:

- 1. An isolated HY2 family bilin reductase comprising an amino acid consensus sequence as illustrated in Figure 5 or in Figure 10 and having bilin reductase activity.
- The bilin reductase of claim 1, wherein said bilin reductase is not hvrccr or atrccr1.
 - 3. The bilin reductase of claim 1, wherein said bilin reductase is not rccr_horvu or rccr_arath.
 - 4. The bilin reductase of claim 1, wherein said bilin reductase is not ycp2_synpy or ycp3_synpy.
 - 5. The bilin reductase of claim 1, wherein said bilin reductase comprises at least 50% sequence conservation as shown in Figure 10.
 - 6. The bilin reductase of claim 1, wherein said bilin reductase comprises at least 70% sequence conservation as shown in Figure 10.
- 7. The bilin reductase of claim 1, wherein said bilin reductase comprises at least 90% sequence conservation as shown in Figure 10.
 - 8. The bilin reductase of claim 1, wherein said bilin reductase comprises at least 80% sequence conservation as shown in Figure 5.
- 9. The bilin reductase of claim 1, wherein said bilin reductase comprises at least 100% sequence conservation as shown in Figure 5.
 - 10. The bilin reductase of claim 1, wherein said bilin reductase is PebA.
 - 11. The bilin reductase of claim 1, wherein said bilin reductase is PebB.
 - 12. A ferredoxin-dependent bilin reductase comprising at least 15% sequence identity with an enzyme selected from the group consisting of HY2_ARATH,

- 5 YCP2_SYNPY, YHP2_PROMA, YHP3_PROMA, YCP3_SYNPY, SLR0116,
 PcyA_ANASP, PcyA_NOSPU, PcyA_SyNY3, PcyA_SYN8.1, PcyA_PROME,
 PebA_SYNPY, PebA_SYN8.1, PebA_PROMA, PebA_PROME, PewbB_NOSPU,
 HY2_ARATH, RCCR_ARATH, and RCCR_HORVU, and where, when aligned with HY2,
 comprises conserved hydrophobic residues at position 137, 157, 158, 256, and 314.
- 13. The bilin reductase of claim 12, wherein said bilin reductase, when aligned with HY2, comprises a residue selected from the group consisting of Pro-151, Phe-221, Ser222, and ASP-171.
 - 14. The bilin reductase of claim 13, wherein said bilin reductase, when aligned with HY2, comprises Pro-151, Phe-221, Ser-222, and ASP-171.
- 15. The bilin reductase of claim 12, wherein said bilin reductase is not hyrccr or atrecr1.
 - 16. The bilin reductase of claim 12, wherein said bilin reductase is not recr_horvu or recr_arath.
- 17. The bilin reductase of claim 12, wherein said bilin reductase is not ycp2_synpy or ycp3_synpy.
 - 18. The bilin reductase of claim 12, wherein said bilin reductase is not HY2.
- An isolated bilin reductase having bilin reductase activity and comprising an amino acid sequence of polypeptide selected from the group consisting of HY2, athy2, slr0116, c362_anab, ycp2-synpy, ycp3_synpy, PcyA_ANASP, PcyA_NOSPU, PcyA_SYNY3, PcyA_SYN81, PcyA_PROME, PebA_SYNPY, PebA_SYN81, PebA_PROMA, PebA_PROME, PebA_NOSPU, PebB_SYNPY, PebB_SYN81, PebB_PROMA, PebB_PROME, PebB_NOSPU, HY2_ARATH, RCCR_ARATH, and RCCR_HORVU, or conservative substitutions thereof.
- 30 20. The bilin reductase of claim 19, wherein said bilin reductase comprises an amino acid sequence of a polypeptide selected from the group consisting of athy2, slr0116, c362_anab, ycp2-synpy, ycp3_synpy, PcyA_ANASP, PcyA_NOSPU,

- PcyA_SYNY3, PcyA_SYN81, PcyA_PROME, PebA_SYNPY, PebA_SYN81, PebA_PROMA, PebA_PROME, PebA_NOSPU, PebB_SYNPY, PebB_SYN81, PebB_PROMA, PebB_PROME, PebB_NOSPU, HY2_ARATH, RCCR_ARATH, and RCCR_HORVU.
- 21. A method of converting a biliverdin to a phytobilin, said method comprising contacting a bilin reductase of claim 1, with a biliverdin whereby said biliverdin is converted to a phytobilin.
 - 22. The method of claim 19, wherein said bilin reductase is a cyanobacterial bilin reductase.
- The method of claim 19, wherein said bilin reductase is an algal bilin reductase.
 - 24. The method of claim 19, wherein said bilin reductase is a plant bilin reductase.
 - 25. The method of claim 21, wherein said bilin reductase is recombinantly expressed.
 - 26. The method of claim 21, wherein said contacting is ex vivo.
 - 27. The method of claim 21, wherein said contacting is in a cell and said bilin reductase is a heterologous polypeptide.
 - 28. The method of claim 21, further comprising contacting said phytochromobilin with a second bilin reductase to produce a phytochrome.
- 25 29. The method of claim 21, further comprising contacting said phytochromobilin with a second bilin reductase to produce a phytofluor.
 - 30. The method of claim 29, wherein said second bilin reductase is PebB.
 - 31. The method of claim 21, wherein said bilin reductase is ycp2-snpy.
 - 32. The method of claim 29, wherein said bilin reductase is ycp3-snpy.

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- 5 33. A nucleic acid comprising a nucleic acid encoding a bilin reductase of any one of claims 1 through 20.
 - 34. The nucleic acid of claim 33, wherein said nucleic acid is a vector.
 - 35. A cell comprising a heterologous nucleic acid comprising a nucleic acid encoding a bilin reductase of any one of claims 1 through 20.
- 36. The cell of claim 35, wherein said cell is selected from the group consisting of an algal cell, a plant cell, a yeast cell, a bacterial cell, an insect cell, and a mammalian cell.
 - 37. A nucleic acid comprising a nucleic acid that specifically hybridizes with a nucleic acid of any one of claims 1 through 20 under stringent conditions and that encodes a polypeptide having bilin reductase activity, wherein said nucleic acid does not encode an hyrcer or an atreer polypeptide.
 - 38. The nucleic acid of claim 37, wherein said nucleic acid is a vector.
 - 39. A method of detecting expression of a polypeptide, said method comprising:

providing a cell comprising a nucleic acid encoding an apophytochrome; and a nucleic acid encoding a bilin reductase that produces a phytobilin that assembles with said apophytochrome to produce a phytofluor;

and detecting an optical signal produced by said phytofluor.

40. A method of producing a photoactive holophytochrome, said method comprising:

co-expressing in a cell:

a heme oxygenase;

an apophytochrome;

and a ferredoxin-dependent bilin reductase;

whereby said cell produces said photoactive holophytochrome and where one or more of said apophytochrome and said ferredoxin-dependent bilin reductase are expressed by heterologous nucleic acids.

- 5 41. The method of claim 40, wherein said cell is selected from the group consisting of an algal cell, a yeast cell, a bacterial cell, a plant cell, an insect cell, and a mammalian cell.
 - 42. The method of claim 40, wherein said ferredoxin-dependent bilin reductase is an HY2 family bilin reductase.
- 10 43. The method of claim 40, wherein said apophytochrome and said ferredoxin-dependent bilin reductase are both expressed by heterologous nucleic acids.
 - 44. The method of claim 40, wherein said heme oxygenase is expressed by a heterologous nucleic acid.
- 45. The method of claim 40, wherein said photoactive holophytochrome 15 is not a phytofluor.
 - 46. The method of claim 45, wherein said ferredoxin-dependent bilin reductase is an HY2 family member.
 - 47. The method of claim 45, wherein said ferredoxin-dependent bilin reductase is *HY2*.
 - 48. The method of claim 45, wherein said ferredoxin-dependent bilin reductase is *pcyA*.
 - 49. The method of claim 40, wherein said photoactive holophytochrome is a phytofluor.
- 50. The method of claim 49, wherein said apophytochrome is expressed as a fusion protein with a protein that is to be labeled with said phytofluor.
 - 51. The method of claim 49, wherein said method comprises expressing the ferredoxin-dependent bilin reductase *pebA* or *pebB*.
 - 52. The method of claim 51, wherein said method comprises expressing both ferredoxin-dependent bilin reductase *pebA* and *pebB*.
- The method of claim 51, wherein said cell is a bacterial cell.

- 5 54. The method of claim 40, wherein said method further comprises recovering said photoactive holophytochrome from said cell.
 - 55. A cell comprising:

a heme oxygenase;

an apophytochrome;

and a ferredoxin-dependent bilin reductase;

whereby said cell produces a photoactive holophytochrome and where one or more of said apophytochrome and said ferredoxin-dependent bilin reductase are expressed by heterologous nucleic acids.

- 56. The cell of claim 55, wherein said cell is selected from the group consisting of an algal cell, a yeast cell, a bacterial cell, a plant cell, an insect cell, and a mammalian cell.
 - 57. The cell of claim 55, wherein said ferredoxin-dependent bilin reductase is an HY2 family bilin reductase.
 - 58. The cell of claim 55, wherein said apophytochrome and said ferredoxin-dependent bilin reductase are both expressed by heterologous nucleic acids.
 - 59. The cell of claim 55, wherein said heme oxygenase is an endogenous heme oxygenase.
 - 60. The cell of claim 55, wherein said heme oxygenase is expressed by a heterologous nucleic acid.
- 25 61. The cell of claim 55, wherein said photoactive holophytochrome is not a phytofluor.
 - 62. The cell of claim 61, wherein said ferredoxin-dependent bilin reductase is an HY2 family member.
- 63. The cell of claim 61, wherein said ferredoxin-dependent bilin reductase is *HY2*.

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- 5 64. The cell of claim 61, wherein said ferredoxin-dependent bilin reductase is *pcyA*.
 - 65. The cell of claim 55, wherein said photoactive holophytochrome is a phytofluor.
- 66. The cell of claim 65, wherein said apophytochrome is expressed as a fusion protein with a protein that is to be labeled with said phytofluor.
 - 67. The cell of claim 65, wherein said cell comprises expressing the ferredoxin-dependent bilin reductase *pebA* or *pebB*.
 - 68. The cell of claim 67, wherein said cell comprises expressing both ferredoxin-dependent bilin reductase *pebA* and *pebB*.
 - 69. The cell of claim 67, wherein said cell is a bacterial cell.
 - 70. A recombinant nucleic acid comprising:

 a nucleic acid encoding a heme oxidoreductase; and
 a nucleic acid encoding and a ferredoxin-dependent bilin reductase;
 where said nucleic acid expresses a functional heme oxidoreducase and a functional bilin reductase.
 - 71. The nucleic acid of claim 70, wherein said heme oxidoreductase and said bilin reductase are under control of the same promoter.
 - 72. The nucleic acid of claim 71, wherein said promoter is a constitutive promoter.
- The nucleic acid of claim 71, wherein said promoter is an inducible promoter.
 - 74. The nucleic acid of claim 71, wherein said promoter is a tissue-specific promoter.
- 75. The nucleic acid of claim 70, wherein said nucleic acid is present in a 30 cell.

- The nucleic acid of claim 75, wherein said cell is selected from the group consisting of an algal cell, a bacterial cell, a plant cell, a yeast cell, a mammalian cell, and an insect cell.
 - 77. The nucleic acid of claim 75, wherein said nucleic acid comprises a gene selected from the group consisting of HO1, HY2, PcyA, PebA, and PebB.
- The nucleic acid of claim 77, wherein said nucleic acid comprises an *HO1* coding region and a *pcyA* coding region.
 - 79. The nucleic acid of claim 78, wherein said nucleic acid further comprises a *pcyB*.